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Research Article
Polymorphism of Insulin-like Growth Factor 1 Gene (*IGF1/Tas*I, *IGF1/Sna*BI, *IGF1/Rsa*I) and the Association with Daily Gain of Pesisir Cattle Local Breed from West Sumatera, Indonesia

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Abstract

Background and Objective: The insulin-like growth factor 1 gene (*IGF1*) is a candidate gene for marker-assisted selection strategies. Two single nucleotide polymorphism of *IGF1* gene namely *IGF1/SnaBI* and *IGF1/TasI* has been reported to be associated with production traits in several cattle breeds. One single nucleotide polymorphism in exon 4 namely *IGF1/Rsa*1 has a significant influence ($p<0.01$) on birth weight, weaning weight and average daily gain of Bali cattle. The objective of this study was to determine the *IGF1/SnaBI*, *IGF1/TasI* and *IGF1/RsaI* polymorphisms and their associations with average daily gain in Pesisir cattle local breed from west Sumatera Indonesia. **Methodology:** The isolation of DNA from 183 blood sample was performed using DNA extraction kit (Promega DNA purification kit) following the manufacture procedure. The amplification of *IGF1* fragment was done by master mix from Thermo Scientific. **Results:** In the case of the *IGF1/SnaBI* polymorphism, three genotypes were observed, frequencies were 0.011, 0.011 and 0.978 for AA, AB and BB, respectively. This gives frequencies of 0.064 and 0.9836 for A and B alleles. For *IGF1-TasI* locus showed that AA genotype occurred with a frequency of 0.978, AC with 0.011 and CC with 0.011 with frequency for allele A and C were 0.9836 and 0.0164, respectively. In the case *IGF1/RsaI* polymorphism produces only TT and CT genotype with frequency 0.027 and 0.973 respectively and frequency of T and C allele were 0.5137 and 0.4863, respectively. **Conclusion:** Among these 3 polymorphism only *IGF1/RsaI* was associated with average daily gain, Thus this polymorphism could be a potential for improving body weight in Pesisir cattle. Association study for *IGF1/SnaBI* and *IGF1/TasI* were strongly limited by low frequency of one allele.

Key words: Pesisir cattle, insulin like growth factor 1, *Sna*BI, *Tas*I, *Rsa*I, PCR-RFLP

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Pesisir cattle breed is one of the existing indigenous cattle breeds in Indonesia that has been adapted to relatively harsh environment, especially to hot and humid climate and low-quality feed to produce meat. Demand for these cattle is high because it is relatively cheaper compared to superior cattle. Recently, selection for better performance of such important indigenous breed has got more attention, especially from the advancement of genetically molecular biotechnology[1]. This is the first research about polymorphism of *IGF1* gene in Pesisir cattle.

The estimated breeding value has been used to select cattle to be sires and dams, which would in many cases result in offspring with improved phenotypic values compared to the previous generation. However, recent studies have focused on molecular genetics to increase the accuracy of genetic selection of young animals especially in carcass traits[2]. In vertebrates, the insulin-like growth factor 1 (*IGF1*) or somatomedin gene plays a key role in various physiological and metabolic processes, where *IGF1* and growth hormone or somatotropin is involved in the somatotropic axis. The *IGF1* is a mediator of many biological effects, for example, it increases the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells and intervenes in the synthesis of DNA, protein, RNA and in cell proliferation[3]. Insulin-like growth factor 1 (*IGF1*) is known to play an important role in various aspects of muscle growth and development[4].

The bovine *IGF1* gene was mapped on chromosome 5, in the centimorgan 73.5[5]. The provisional nucleotide sequence is approximately 72 kb (ID number 281239) it is consist 6 exon. Due to its biological function, the *IGF1* gene is considered to be a candidate gene for predicting growth and meat quality traits in animal genetic improvement schemes[6,7]. The bovine *IGF1* gene has two polymorphisms located in the promoter region: A CAn microsatellite[8] and a T/C transition, also known as the Single Nucleotide Polymorphism (SNP) *IGF1*/*Sna*BI[9,10]. The microsatellite has been associated with birth weight and weaning in Hereford cattle, but these associations were not found in breeds such as Nellore, Canchim and Simmental/Angus crosses studied[11]. After its evaluation with growth characteristics in Angus cattle, the SNP *IGF1*/*Sna*BI was considered to be a potential molecular marker associated with weight gain during the first 20 days after weaning[9]. Curi *et al.*[12] studied some Nellore, Canchim and several population crosses and found a significant association ($p<0.05$)of SNP with body weight and subcutaneous back fat. Li *et al.*[13] analyzed the association between SNP and growth characteristics and found an unstable association between the BB genotype with birth weight, average daily gain and average daily pre-weaning in Beefbooster cattle. Although there is no evidence of a functional role in gene expression, these polymorphisms are considered to be potential molecular markers[14] and it has even been proposed that the existence of other polymorphic sites linked with these markers will help to explain the effect of the *IGF1* gene on phenotypic characteristics. Szewczuk *et al.*[15] found a significant association ($p<0.05$) of *IGF1*/*Tas*I polymorphism with milk production, fat and protein yield where in the 2nd and 3rd lactations where cows carrying the CC genotype produced more milk than the AA individuals. In this study, the effect of the SNP *IGF1*/*Sna*BI on growth characteristics was determined in Mexican beef cattle.

Siadkowska *et al.*[14] observed the effect of polymorphism in the 5'-non-coding region of the *IGF1* gene on production traits in Polish Holstein Friesian cattle. Mullen *et al.*[16] identified a total of 16 SNPs in *IGF1* and GH gene and its association with milk production, body condition score and fertility traits in Holstein Friesian lactating dairy cows. Szewczuk *et al.*[15] recorded associations between *IGF1*/*Tas*I polymorphism in Polish Holstein Friesian cows and its association with milk traits and Nicolini *et al.*[17] observed polymorphism in *IGF1* gene and its association with postpartum resumption of ovarian cyclicity in Holstein cows. Maskur *et al.*[18] identified T/C transition in exon 4 *IGF1* gene in bali cattle, which can be identified using *Rsa*I restriction enzyme. Genotypic polymorphisms in the *IGF1* polymorphism of *IGF1*/*Rsa*I has a significant influence ($p<0.01$) on birth weight, weaning weight and average daily gain of Bali cattle[18]. Polymorphism of *IGF1* in Pesisir cattle is not yet done. The aim of this study was to observe polymorphism of *IGF1* in Pesisir cattle and its relation to average daily gain.

MATERIALS AND METHODS

Animal: One hundred and eighty three Pesisir cattle of 1.5 years old were used in this study. Pesisir cattle were reared under pastoral condition in Pesisir Selatan district, West Sumatera, Indonesia. These samples were composed of 117 males and 66 females. The average of daily gain was measured by weighing the cattle for 3 months period.

DNA extraction: Blood samples for DNA genotyping were collected from the jugular vein using vacuum tubes containing EDTA. The isolation of DNA from whole blood was performed using Wizard® Genomic DNA purification kit

(Promega Corporation, Madison, WI, USA) following the manufacture protocol.

PCR amplification of *IGF1* gene: The promoter region and exon 4 regions of the *IGF1* genes were amplificated using the Polymerase Chain Reaction (PCR). The following primers were used for amplification of the *IGF1*/*Sna*BI fragment forward 5′–ATT-ACA-AAG-CTG-CCT-GCC-CC–3′ and reverse 5′–ACC-TTA-CCC-GTA-TGA-AAG-GAA-TAT-ACG-T–3′ as described by Ge *et al.*[10]. For the *IGF1*/*Tas*I analysis, the primer sequences uses designed by Zych *et al.*[19] forward 5′-TCA-TCC-AGC-TGA-GAG-ATT-TGA-AT-3′ and reverse 5′-TGT-GTG-TGT-GTG-TGT-GTG-TGA-AT-3′. For *IGF1*/*Rsa*I analysis, the primer sequences designed by Maskur *et al.*[18] were forward 5′-CCACTCTAAAGCTAGGCCTCTCTC-3′ and reserve 5′-GAA-GTC-TAT-GAG-GGT-ATG-AAT-3′.

The PCR was performed in a reaction volume of 25 μL using 25 ng of genomic DNA of each sample, 20 pmol of each primer, 12.5 μL master mix (Thermo scientific, Lithuania) and 6.5 μL nuclease free water. Thermal cycling (Eppendorf Thermocycler Master cycler Gradient) was carried out by initial denaturation at 94°C for 4 min, followed by 34 cycles each at 94°C for 45 sec, annealing temperature for 45 sec (62°C for *IGF1*/*Sna*BI, 58°C for *IGF1*/*Tas*I and 62°C for *IGF1*/*Rsa*BI) and elongation at 72°C for 1 min. After 35 cycles, a final extension was given at 72°C for 10 min. The amplified DNA fragments were separated on 2% agarose gel (2 g agarose in 100 mL 1× TBE (89 mM tris-HCl, 2.5 mM EDTA and 89 mM boric acid, pH 8.3)), stained with ethidium bromide, visualized on a UV trans illuminator and photographed by gel documentation system (Thermo Scientific).

The PCR fragments of the *IGF1* genes were analyzed by RFLP, with digestion of the fragments with *SnaB*I, *Tas*I and *Rsa*BI (Thermo Scientific, Lithuania) restriction May 24, 2017respectively, at 37°C for 8 h. The cleaved fragments were separated by electrophoresis on 2.0% agarose gel in 1× TBE buffer (89 mM tris-HCl, 2.5 mM EDTA and 89 mM boric acid, pH 8.3) containing 0.05 μg mL^{-1} ethidium bromide at 75 V for 2 h. The bands were visualized under ultraviolet light in a gel-doc system (Thermo Scientific).

The PCR products of 249 bp of *IGF1*/*Sna*BI were digested with 5U of the *Sna*BI endonuclease (37°C/6 h) (Thermo Scientific, Lithuania) which recognized TAC↓GTA sequence within the PCR product. For the *IGF1*/*Tas*I analysis 146 bp fragment were digested with the 5U of the *Tas*I restriction enzyme (65°C/6 h) (Thermo scientific, Lithuania) which recognized ↓AATT. For *IGF1*-*Rsa*I analysis fragment 368 bp fragment were digested with the 5 U of the *Rsa*I restriction enzyme (65°C/8 h) (Thermo Scientific, Lithuania) which recognized GT↓AC.

Statistical analysis: Genotypes of individual animals were recorded by direct counting of the bands. The allelic and genotypic frequencies were calculated. The association between the genotype of the *IGF1* gene and carcass traits was analyzed using a General Linear Model (GLM) procedure[20]. The model is given as Eq. 1:

$$y_{ijk} = \mu + G_i + F_j + e_{ijk} \quad (1)$$

where, y_{ijk} is the observation of average of daily gain, μ is the overall mean of population, G_i is the effect of sex, F_j is the fixed effect of *IGF1* genotypes (j = 1, 2, 3) and e_{ijk} is the random of residual error assumed as a normal distribution with a mean of 0 and a variance of σ^2.

RESULTS

Amplification using primer *IGF1*-*Sna*B1 were amplified to produce a 249 bp fragment. The amplified fragment were digested with *Eco*105I (*SnaB*I) restriction endonuclease and then subjected to electrophoretic separation in ethidium bromide stained 2.0% agarose gel resulted in 3 fragments (249, 226 and 23 bp) (Fig. 1).

Genotype AA was characterized by the presence of two restriction fragments of 226 and 23 bp, while genotype BB was determined by the presence of a single 249 bp. Heterozygous genotype AB showed three fragments of 246, 226 and 23 bp. The 226 bp fragment and the 249 bp whole fragment were clearly visible, while the 23 bp fragment not visible since migrated to the base of the gel. The distribution of genotypes of the *IGF1* gene are presented below. Three genotypes were observed, frequencies were 0.011, 0.011 and 0.978 for AA, AB and BB, respectively. This gives frequencies of 0.0164 and 0.983 for A and B alleles (Table 1).

A transversion of A (allele A) to C (allele C) in the P1 promoter region of *IGF1* gene was identified using ACRS-PCR method. Primer *IGF1*-*Tas*IR introduced an artificial *Tas*I restriction site. Amplification using these primer were

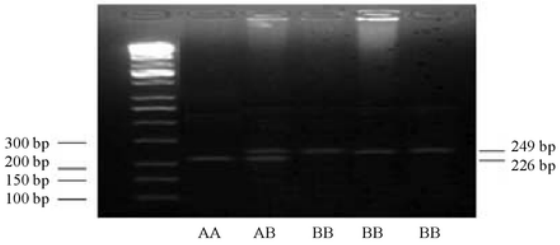

Fig. 1: Electrophoresis of the *IGF1* gene fragment digested with *Sna*BI 0n 2% agarose gel

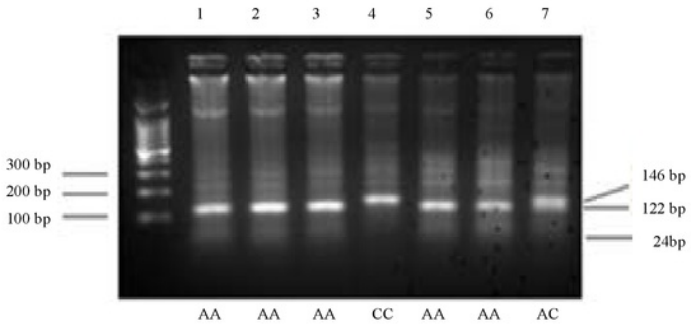

Fig. 2: Electrophoresis of the *IGF1* gene fragment digested with *Tas*I on 2% agarose gel

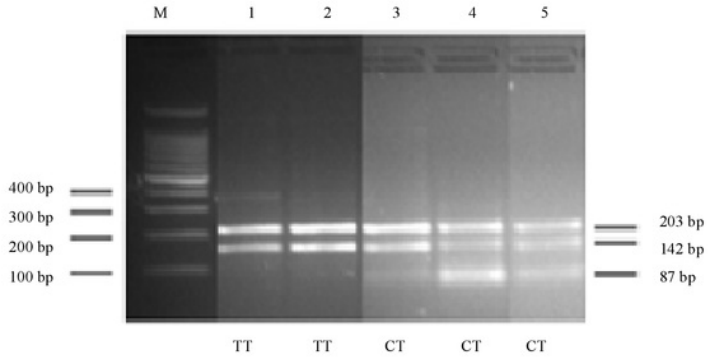

Fig. 3: Electrophoresis of the *IGF1* gene fragment digested with *Rsa*I on 2% agarose gel

Table 1: Allelic and genotypic frequencies of the *IGF1* in Pesisir cattle

Genes	N	Genotypic observation and genotypic frequency			Allele frequency χ^2 (HWE)	
		AA	AB	BB	A	B
IGF1/*Sna*BI 80.15**	183	2 (0.011)	2 (0.011)	179 (0.978)	0.0164	0.9836
		AA	AC	CC	A	C
IGF1/*Tas*I 80.15**	183	179 (0.978)	2 (0.011)	2 (0.011)	0.9836	0.0164
		CC	CT	TT	C	T
IGF1/*Rsa*I 164.13**	183	0 (0)	178 (0.973)	5 (0.027)	0.4863	0.5137

**p<0.01, HWE: Hardy-Weinberg equilibrium

amplified *IGF1* gene to produce a 146 bp fragment. Digestion of the 146 bp PCR product with *Tas*I restriction endonuclease and then subjected to electrophoretic separation in ethidium bromide stained 2.0% agarose gel resulted in 2 DNA bands (122 and 24 bp) for homozygote AA and three bands (146, 122 and 24 bp) for the AC heterozygote (Fig. 2). The DNA amplified from homozygous CC animals remained undigested with *Tas*I restriction enzyme. The AA genotype occurred with a frequency of 0.978, AC with 0.011 and CC with 0.011 with frequency for allele A and C were 0.9836 and 0.0164, respectively (Table 1).

Amplification using primer *IGF1*/*Rsa*I were amplified to produce a 345 bp fragment in exon 4. The point mutations in exon 4 changed the amino acid sequence of *IGF1* in which methionine (AUG) changed to threonine (ACG)[18]. These mutations can be identified using restriction enzyme *Rsa*I (Fig. 3). According to Maskur *et al.*[18] the digestion using *Rsa*I produces two alleles, namely T and C alleles. T allele which is not being cut by *Rsa*I, produced two DNA fragments: 203 and 142 bp, while C allele which is being cut by *Rsa*I, produced three DNA fragments: 203, 87 and 55 bp. The digestion of the *IGF1* locus occurred because of mutations that cause *Rsa*I enzyme recognized the sequence (GT↓AC) as the site of cutting. In Pesisir cattle digesting these fragment with *Rsa*I enzyme produces only TT and CT genotype (Fig. 3) with frequency 0.027 and 0.973, respectively and frequency allele T and C were 0.4863 and 0.5137, respectively (Table 1).

Table 2: Average daily gain (kg day^{-1}) in Pesisir cattle base on genotype *IGF1*

Genes	Genotype average daily gain (kg day^{-1})		
IGF1/*Sna*BI	AA	AB	BB
	0.14003	0.14004	0.13999
IGF1/*Tas*I	AA	AC	CC
	0.14001	0.13999	0.1398
IGF1/*Rsa*I	CC	CT	TT
	-	0.1647[a]	0.1390[b]

Mean in the same row with different superscript differ significantly at p<0.05

DISCUSSION

In the present study the polymorphism of the *IGF1* gene was examined. Previous studies by Ge *et al.*[10], Szewczuk *et al.*[15,21] and Maskur *et al.*[18], shown that the *IGF1*/*Tas*I, *IGF1*/*Sna*BI, *IGF1*/*Rsa*I were polymorphic and were association with production trait.

For *IGF1*/*Sna*BI 3 genotypes AA, AB, BB were observed with frequencies 0.0125, 0.0125 and 0.9750, respectively and the frequencies of allele A and B were 0.00625 and 0.99375, respectively. These result almost the same with research by Laureano *et al.*[22] who found genotypes AB and BB at frequencies of 0.02 and 0.98, respectively in Nellore heifers. These result also almost the same with research by Szewczuk *et al.*[21] who found frequency of genotypes AB and BB detected by RFLP was 0.02 and 0.98, respectively. Curi *et al.*[12] and Akis *et al.*[23] reported similar result. The frequencies of the B allele were almost fixed in South Anatolian Red (0.82), East Anatolian Red (0.87) and Beef master population (0.97) and fixed in Nellore population (1.00). The B allele is a main characteristic in *Bos indicus* or Zebu cattle. Thus, the high frequency of the Ballele in the current population could have resulted from the breed compositions of Zebu cattle, since Pesisir cattle close related to Zebu cattle[1]. But the present result are in contrast compare to Ge *et al.*[10] who studying the same IGF-1 gene polymorphism in 760 Angus calves, detected three different genotypes (AA: 0.43; AB: 0.42; BB: 0.15) and observed a significant association (p<0.01) between this RFLP and growth traits while in these study no association between this RFLP with average daily gain (Table 2). However, in the several study, cows with the A allele were always significantly associated (p<0.01) with a higher milk, fat and protein yield[14,24]. In contrast, no association was found between *IGF1* RFLP-*Sna*BI and dairy production traits in Holstein cattle[25]. The population is not hardy-weinberg equilibrium. It is possible that this gene has not exposed to selection.

For *IGF1*/*Sna*BI 3 genotypes AA, AC, CC were observed with frequencies 0.978, 0.011 and 0.0,011 respectively and the frequencies of allele A and C were 0.9836 and 0.0.0164, respectively and observed no association between this RFLP with average daily gain (Table 2). These result almost the same trend with research by Zych *et al.*[19] who found 3 genotypes AA, AC and CC with frequency were 0.723, 0,236 and 0.041, respectively and the of allele A and C were 0.841 and 0.159.

There are contrasting allelic frequencies distributions between T and C allele in *IGF1-Rsa*1 gene of Pesisir cattle where frequency of T allele was higher than the frequency of C allele with the frequencies were 0.5137 and 0.4863, respectively. These result in contrast with allele frequency in Bali cattle where C allele higher than T allele[18] where the frequency allele C and allele T where 0.824 and 0.176, respectively. Higher allele T also reported by De la Rosa Reyna *et al.*[26] who found that the T/C transition in intron 4 produced T and C allele with frequency 0.70 and 0.30 in Beefmaster cattle and 0.52 and 0.48 in Charolais cattle. T/C transition in the promoter region (SNP IGF-1/SnaB1) was equal to the T/C transition in intron 4 in which the frequency of C allele was higher than T allele. The frequency of C allele was 0.97 in Beefmaster cattle and 0.74 in Charolais cattle. The C allele is a characteristic of *Bos indicus* cattle because of the existence of a highly conservative C allele in Nellore cattle population[5].

The RFLP-*Rsa*I, 250 Balinesse cattle were genotyped at T/C polymorphism with frequency TT-0.136, CT-0.080 and CC-0.784[18]. The genotype frequencies at polymorphic loci of exon 4 *IGF1* gene showed a highly significant difference (p<0.01). Where CC genotype had average daily gain of higher than the CT and CT genotype and CT genotype was superior than TT genotype. while in Pesisir cattle CT genotype had average daily gain than TT genotype. Higher allele T in Pesisir cattle in line with the fact that Pesisir cattle is the second small cattle in the word.

The study was limited by small sample that observed low frequency of one allele, so association study can not be done for *IGF1*/*Sna*BI and *IGF1*/*Tas*I. Association *IGF1*/*Rsa*I with average daily gain will advance the strategy for improving Pesisir cattle by using this new maker.

CONCLUSION AND FUTURE RECOMMENDATION

The results of this study indicated that *IGF1*/*Sna*BI and *IGF1*/*Tas*I polymorphism were not in association with average daily gain, only *IGF1*/*Rsa*I polymorphism was associated with average daily gain in Pesisir cattle. Then this marker can be used to improve body weight in Pesisir cattle. More tests are needed for large populations to verify the associated effects of these three polymorphisms as the potential genetic markers.

SIGNIFICANCE STATEMENTS

The Pesisir cattle is one of the indigenous cattle breeds in Indonesia that characterized by small body weight. By knowing the association of *IGF1*/*Rsa*I with average daily gain and by using this new marker will advanced the strategy for improving Pesisir cattle.

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